

SEQUENCE LISTING

<110> Goshorn, Stephen C.
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Reno, Jonh M.

<120> STREPTAVIDIN EXPRESSED GENE FUSIONS AND
METHODS OF USE THEREOF

<130> 690022.547

<140> US
<141> 2000-06-05

<160> 46

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<212> DNA
<213> Streptomyces avidinii

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cgaggcgagg atcaacacccc agtggctgt gacccggcgc accaccgagg ccaacgcctg 480
gaagtccacg ctggcggcc acgacaccc ttccaaagggtg aagccgtccg ccgcctccat 540
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<213> Streptomyces avidinii

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Gln Val Ser Ala Ala Glu Ala Gly Ile Thr Gly Thr Trp Tyr Asn Gln
35 40 45
Leu Gly Ser Thr Phe Ile Val Thr Ala Gly Ala Asp Gly Ala Leu Thr

50	55	60
Gly	Thr	Tyr
Thr	Tyr	Glu
Gly	Ser	Ala
Asn	Val	Gly
Asn	Ala	Ale
Glu	Ser	Glu
Arg	Arg	Arg
Tyr	Tyr	Tyr
Asp	Ser	Asn
Pro	Ala	Ala
Thr	Ala	Asn
Asn	Trp	Gly
Asn	Trp	Asn
Tyr	Val	Tyr
Arg	Asn	Asn
Asn	His	Leu
His	Ser	
Leu	Gly	
Trp	Trp	
Trp	Val	
Ala	Trp	
Lys	Lys	
Asn	Asn	
Asn	Ala	
Ala	Arg	
Arg	Ile	
Ile		
Ala	Thr	
Thr	Trp	
Trp	Ser	
Ser	Gly	
Gly	Gln	
Gln	Tyr	
Tyr	Val	
Val	Gly	
Gly	Ala	
Ala	Glu	
Glu	Ala	
Ala	Arg	
Arg	Ile	
Ile		
Ala	Thr	
Thr	Trp	
Trp	Leu	
Leu	Leu	
Leu	Thr	
Thr	Ser	
Ser	Gly	
Gly	Thr	
Thr	Glu	
Glu	Ala	
Ala	Asn	
Asn	Ala	
Ala	Trp	
Trp	Leu	
Leu	Leu	
Leu	Thr	
Thr	Ser	
Ser	Gly	
Gly	Thr	
Thr	Glu	
Glu	Ala	
Ala	Asn	
Asn	Ala	
Ala	Arg	
Arg	Ile	
Ile		
Ala	Asn	
Asn	Ala	
Ala	Asn	
Asn	Asn	
Asn	Gly	
Gly	Val	
Val	Asn	
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cggccactcc	gegaccacgt	ggagcggcca	gtacgtcg	ggcgccgagg	cgaggatcaa	1440
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<210> 4
<211> 431

<212> PRT

<213> Streptomyces avidinii

<400> 4

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 Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
 35 40 45
 Gln Gly Ile Arg Gly Asn Leu Asp Trp Tyr Gln Gln Lys Pro Gly Lys
 50 55 60
 Gly Pro Lys Leu Leu Ile Tyr Ser Thr Ser Asn Leu Asn Ser Gly Val
 65 70 75 80
 Pro Ser Arg Phe Ser Gly Ser Gly Ser Asp Tyr Thr Leu Thr
 85 90 95
 Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln
 100 105 110
 Arg Asn Ala Tyr Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile
 115 120 125
 Lys Ile Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly
 130 135 140
 Gly Ser Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys
 145 150 155 160
 Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Phe Asn Ile
 165 170 175
 Lys Asp Thr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
 180 185 190
 Gln Trp Met Gly Arg Ile Asp Pro Ala Asn Gly Asn Thr Lys Ser Asp
 195 200 205
 Leu Ser Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Thr Ser Ile Asn
 210 215 220
 Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Asp Asp Thr Ala Val
 225 230 235 240
 Tyr Tyr Cys Ser Arg Glu Val Leu Thr Gly Thr Trp Ser Leu Asp Tyr
 245 250 255
 Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Ser Gly Ser Ala
 260 265 270
 Asp Pro Ser Lys Asp Ser Lys Ala Gln Val Ser Ala Ala Glu Ala Gly
 275 280 285
 Ile Thr Gly Thr Trp Tyr Asn Gln Leu Gly Ser Thr Phe Ile Val Thr
 290 295 300
 Ala Gly Ala Asp Gly Ala Leu Thr Gly Thr Tyr Glu Ser Ala Val Gly
 305 310 315 320
 Asn Ala Glu Ser Arg Tyr Val Leu Thr Gly Arg Tyr Asp Ser Ala Pro
 325 330 335
 Ala Thr Asp Gly Ser Gly Thr Ala Leu Gly Trp Thr Val Ala Trp Lys
 340 345 350
 Asn Asn Tyr Arg Asn Ala His Ser Ala Thr Thr Trp Ser Gly Gln Tyr
 355 360 365
 Val Gly Gly Ala Glu Ala Arg Ile Asn Thr Gln Trp Leu Leu Thr Ser
 370 375 380
 Gly Thr Thr Glu Ala Asn Ala Trp Lys Ser Thr Leu Val Gly His Asp
 385 390 395 400

115	120	125
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130	135	140
Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Asn		
145	150	155
Met His Trp Val Lys Gln Thr Pro Gly Gln Gly Leu Glu Trp Ile Gly		160
165	170	175
Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe Lys		
180	185	190
Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met		
195	200	205
Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala		
210	215	220
Arg Ala Gln Leu Arg Pro Asn Tyr Trp Tyr Phe Asp Val Trp Gly Ala		
225	230	235
Gly Thr Thr Val Thr Val Ser Ser Gly Ser Gly Ser Ala Asp Pro Ser		240
245	250	255
Lys Asp Ser Lys Ala Gln Val Ser Ala Ala Glu Ala Gly Ile Thr Gly		
260	265	270
Thr Trp Tyr Asn Gln Leu Gly Ser Thr Phe Ile Val Thr Ala Gly Ala		
275	280	285
Asp Gly Ala Leu Thr Gly Thr Tyr Glu Ser Ala Val Gly Asn Ala Glu		
290	295	300
Ser Arg Tyr Val Leu Thr Gly Arg Tyr Asp Ser Ala Pro Ala Thr Asp		
305	310	315
Gly Ser Gly Thr Ala Leu Gly Trp Thr Val Ala Trp Lys Asn Asn Tyr		320
325	330	335
Arg Asn Ala His Ser Ala Thr Thr Trp Ser Gly Gln Tyr Val Gly Gly		
340	345	350
Ala Glu Ala Arg Ile Asn Thr Gln Trp Leu Leu Thr Ser Gly Thr Thr		
355	360	365
Glu Ala Asn Ala Trp Lys Ser Thr Leu Val Gly His Asp Thr Phe Thr		
370	375	380
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<211> 1280

<212> DNA
<213> Streptomyces avidinii

<400> 7

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cttcctacaa tcagaagttc aaaggcaagg ccacattgac tgccagacaaa tcctccagca	240
cagcctacat gcagctcagc agcctgacat ctgaggactc tgccgtctat tactgtgcaa	300
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ccgtgagcaa gatctctggt ggcggtggt cggccgggt tgggtcggtt ggcggcggct	420
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caatcctgttc tgcacatctcca ggggagaagg tcacaatgac ttgcagggcc agctcaagtg	540
taagttacat gcactggtaa cagcagaagc caggatcctc ccccaaacc cttgatttatg	600

ccacatccaa cctggttct ggagtccctg ctcgcttcag tggcagtggg tctggacct	660
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<210> 8
<211> 423
<212> PRT
<213> Streptomyces avidinii

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35 40 45	
Trp Ile Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln	
50 55 60	
Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr	
65 70 75 80	
Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr	
85 90 95	
Tyr Cys Ala Arg Ala Gln Leu Arg Pro Asn Tyr Trp Tyr Phe Asp Val	
100 105 110	
Trp Gly Ala Gly Thr Thr Val Thr Val Ser Lys Ile Ser Gly Gly Gly	
115 120 125	
Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Gly Gly	
130 135 140	
Ser Gly Gly Gly Ser Ser Asp Ile Val Leu Ser Gln Ser Pro Ala	
145 150 155 160	
Ile Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg Ala	
165 170 175	
Ser Ser Ser Val Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Ser	
180 185 190	
Ser Pro Lys Pro Trp Ile Tyr Ala Thr Ser Asn Leu Ala Ser Gly Val	
195 200 205	
Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr	
210 215 220	
Ile Ser Arg Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln	
225 230 235 240	
Trp Ile Ser Asn Pro Pro Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu	
245 250 255	
Lys Ser Ser Gly Ser Gly Ser Ala Asp Pro Ser Lys Asp Ser Lys Ala	
260 265 270	
Gln Val Ser Ala Ala Glu Ala Gly Ile Thr Gly Thr Trp Tyr Asn Gln	

275	280	285
Leu	Gly	Ser
Thr	Phe	Ile
Ile	Val	Thr
Gly	Ala	Gly
Asp	Ala	Gly
Ala	Leu	Thr
290	295	300
Gly	Thr	Tyr
Glu	Ser	Ala
Val	Gly	Asn
Ala	Ser	Glu
Arg	Tyr	Ser
Asp	Ser	Ala
Pro	Ala	Thr
305	310	315
Thr	Gly	Arg
Arg	Tyr	Tyr
Tyr	Asp	Asp
Asp	Ser	Gly
Ser	Gly	Ser
Ala	Thr	Thr
Trp	Val	Ala
Trp	Ala	Trp
Lys	Asn	Asn
Asn	Tyr	Tyr
Tyr	Arg	Arg
Arg	Asn	Ala
His	His	Ser
325	330	335
Leu	Gly	Trp
Gly	Trp	Thr
Thr	Val	Ala
Trp	Trp	Trp
Lys	Asn	Asn
Asn	Tyr	Arg
Arg	Asn	Ala
His	Ser	350
340	345	
Ala	Thr	Thr
Thr	Trp	Ser
Ser	Gly	Gln
Gln	Tyr	Tyr
Tyr	Val	Gly
Gly	Ala	Glu
Ala	Glu	Ala
Arg	Ile	Arg
355	360	365
Asn	Thr	Gln
Gln	Trp	Trp
Trp	Leu	Leu
Leu	Thr	Ser
Ser	Gly	Thr
Gly	Thr	Thr
Thr	Glu	Gly
Glu	Ala	Ala
Ala	Asn	Asn
Asn	Ala	Trp
370	375	380
Lys	Ser	Thr
Thr	Leu	Val
Leu	Gly	His
Gly	Asp	Thr
Asp	Thr	Phe
Phe	Thr	Lys
Lys	Val	Val
Val	Lys	Lys
385	390	395
Ala	Ala	Ala
Ser	Ile	Asp
Ile	Asp	Ala
Ala	Lys	Lys
Lys	Ala	Gly
Ala	Gly	Val
Gly	Val	Asn
Asn	Asn	Gly
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Pro	Leu	Asp
Asp	Ala	Val
Ala	Gln	Gln
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<210> 9

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> pKOD linker

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Gly Ser

<210> 10

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Linker used to create a scFvSA version of
anti-CD20mAb, B9E9 in the VLVH orientation

<400> 10

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<210> 11

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<223> Linker used to create a version of B9E9 scFvSA in

the VHVL orientation

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 1 5 10 15
 Gly Gly Gly Ser Gly Gly Gly Ser
 20 25

<210> 12
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<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer

<400> 12
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32

<210> 13
<211> 31
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<213> Artificial Sequence

<220>
<223> Oligonucleotide primer

<400> 13
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31

<210> 14
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<223> Oligonucleotide primer

<400> 14
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<210> 15
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<213> Artificial Sequence

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34

<210> 16
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<212> DNA
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<220>
<223> Oligonucleotide primer

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<210> 17
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<400> 17
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<212> DNA
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<400> 18
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<210> 19
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<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer

<400> 19
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<210> 20
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<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer

<400> 20
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<210> 21

<211> 58
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer

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<210> 22
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<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer

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<210> 23
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<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer

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<210> 24
<211> 32
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<213> Artificial Sequence

<220>
<223> Oligonucleotide primer

<400> 24
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<210> 25
<211> 35
<212> DNA
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<220>
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<210> 26
 <211> 35
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Oligonucleotide primer

<400> 26
 agaccagaga tcttgctcac ggtgaccgtg gtccc 35

<210> 27
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 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Oligonucleotide primer

<400> 27
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 tgggtcggtt ggccggccgtt 79

<210> 28
 <211> 79
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Oligonucleotide primer

<400> 28
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<210> 29
 <211> 18
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Linker sequence

<400> 29
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 1 5 10 15
 Gly Ser

<210> 30
 <211> 35
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Linker sequence

<400> 30

Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly
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Gly Gly Gly Ser Gly Gly Ser Gly Gly Gly Ser Gly Gly
20 25 30
Gly Gly Ser
35

<210> 31

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Linker sequence pKOD2

<400> 31

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1 5 10 15
Asp Ser

<210> 32

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer

<400> 32

acgacggttt ctgcggcggt c

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<210> 33

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer

<400> 33

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<210> 34

<211> 33

<212> DNA

<213> Artificial Sequence

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<223> Oligonucleotide primer

<400> 34	ggatccaagg ttacgatcac ggtcatgaac acg	33
<210> 35		
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<213> Streptomyces avidinii		
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<210> 41

<211> 768

<212> DNA

<213> Streptomyces avidinii

<400> 41

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ggctcgagcg	atattgtat	gtcacagtc	ccgtcctccc	taccgggtc	agttggcgaa	480
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<210> 42

<211> 765

<212> DNA

<213> Streptomyces avidinii

<400> 42

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<211> 741

<212> DNA

<213> Streptomyces avidinii

<400> 43

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<210> 44

<211> 765

<212> DNA

<213> Streptomyces avidinii

<400> 44

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<210> 45

<211> 765

<212> DNA

<213> Streptomyces avidinii

<400> 45

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<212> DNA
<213> Streptomyces avidinii

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